RESEARCH PLAN PROPOSAL

ANTIOXIDANT AND ANTIMUTAGENIC POTENTIAL OF AJWAIN (*Trachyspermum ammi)* AND FENNEL (*Foeniculum vulgare)* SEEDS AGAINST INDUCED MUTAGENESIS

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INTRODUCTION

Cancer is a major leading cause of death worldwide. Chemically induced carcinogenesis consists of three distinct stages: initiation, promotion and progression (Moolgavkar, 1978). DNA mutation plays a major role in the initiation stage, and is usually both rapid and irreversible (De Flora, 2005). Both reduction of carcinogen exposure in the environment and increase in chemopreventive agent intake are of prime importance for reducing cancer incidence in humans. Potential antimutagens and anticarcinogens from natural products include flavonoids, a common group of polyphenolic compounds that are ubiquitous in nature. They have been reported to have antiviral, anti-allergic, anti-platelet, antiinflammatory, antitumor and antioxidant activities. Most of mutagenic and carcinogen agents display their destructive effects through free radicals including reactive oxygen's species (ROS). ROS have a role in etiology of diseases such as cancer, cardiocellular, nerves problems and senescence. So daily consumption of antioxidants enhances immunity of body against free radicals production and serves as anticancer agents. Extensive research in the last few decades on the detection and characterization of antimutagenic compounds from edible, non-edible and medicinal plants and marine organisms has demonstrated a great diversity. Several authors have suggested that natural antimutagens may belong to class of flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins and several others all of which are secondary plant metabolites. More than 500 compounds belonging to at least 25 chemical classes have been recognized as possessing antimutagenic/protective effects (Boone *et al,* 1990). In recent years, there has been an increased interest in identifying the antimutagenic and anticarcinogenic constituents of both dietary and medicinal plants all over the world.

Trachyspermum ammi commonly known as 'Ajwain' is distributed throughout India and is mostly cultivated in Gujarat and Rajasthan. Medicinally, it has been proven to possess various pharmacological activities like antimicrobial (Khanuja, 2004), hypolipidemic (Javed *et al,* 2002), antihypertensive (Gilani *et al,*2005), antispasmodic (Gilani *et al* ,2005), antilithiasis and diuretic (Ahsan *et al,* 1990), abortifacient (Kaur,1998), antitussive (Boskabady *et al,* 2005), nematicidal (Murthy *et al,* 2009), anthelmintic (Kwon *et al,* 2007) and antifilarial (Mathew *et al,* 2008) activity.

Fennel (*Foeniculum vulgare* Mill) and ajwain (*Trachyspermum ammi)* both are well known Umbeliferous plants from Apiaceae family. Fennel is chiefly known as culinary herb but it is a commonly used household remedy for various medicinal purposes such as anticancer (Pradhan *et al, 2008),* Antibacterial (Kaur and Arora, 2008), Antifungal (Pai *et al,* 2010), antihirsutism (Javidnia *et al,* 2003), anti-inflammatory (Choi and Hwang, 2004), Hepatoprotective (Ozbek *et al,* 2003) activities.

In the Present study we aim to determine the antioxidant and antimutagenic potential of ajwain (*Trachyspermum ammi) and* Fennel (*Foeniculum vulgare* Mill) seeds.

REVIEW OF LITERATURE

World over, the general opinion is tilting towards use of herbal drugs. The gradual rise in trade of these drugs stands testimony to this. Consequently, in recent years there is a much focus on replacing synthetic food additives which might have adverse effects with those of plant-based natural ones (Descalzo and Sancho, 2008). The increasing uses of herbal products demand extra attention with particular focus on their safety, effectiveness and drug interactions.

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin (Cowan, 1999). Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years (Rios, 2005).

Herbalists generally use unpurified plant extracts containing several different constituents. It is claimed that these can work together synergistically so that the effect of the whole herb is greater than the summed effects of its components (Sanjoy *et al,* 2003). Research is now in progress to explore the applications of some essential oils for therapeutic uses and management of infectious diseases as an alternative to standard drugs remedies (Sokovic and Van Griensven, 2006; Bozin *et al,* 2006; Celiktas *et al,* 2007; Politeo *et al,* 2007; Kelen & Tepe, 2008;).

Spices have also been used since antiquity to preserve food due to the presence of antioxidant phytochemicals (Lee *et al,* 2010).

Over the last few decades, a substantial body of scientific evidence is available demonstrating wide range of pharmacological and nutraceutical activities of medicinal herbs (Burt, 2004; Celiktas *et al,* 2007; Edris, 2007). These include antioxidant, anticancer, anti-inflammatory activities.

Studies of human cancer have established that a mutagenic event is very likely the initiating factor in some kinds of cancers. Most known carcinogenic chemicals and radiations also are mutagenic. Therefore, demonstration of mutagenic activity suggests that the substance may be (but need not be) carcinogenic. The common association between mutagenic activity and carcinogenicity is the basis for using short-term mutagenesis tests with bacteria or cultured cells to detect potential carcinogens (Ames, 1983). A plethora of synthetic and natural substances apart from various genotoxic, physical and biological agents are known to act as mutagenic, co carcinogenic and/or carcinogenic agents.(Mitscher *et al,*1986). Some of these are sodium azide, ethidium bromide, hydroxylamine, 2 aminofluorine, benzo(a)pyrene etc.

These mutagens and carcinogens act through the generation of Reactive Oxygen Species (ROS). ROS play a major role as endogenous initiators of degenerative process, such as DNA damage and mutation (and promotion) that may be related to cancer and heart disease and aging (Ames,1983). The dietary antioxidants present in many fruits,spices, vegetables and tea had improved an immune status, scavenge free radicals, reduce the production of DNA adducts and could be of effective means in preventing variety of diseases (Van Breda *et al,* 2005, Devaraj *et al,* 2008).

Since, the mutagens are involved in the initiation and promotion of several human diseases, including cancer, the significance of novel bioactive phytocompounds in

counteracting these pro-mutagenic and carcinogenic effects is now gaining credence. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are referred to as antimutagens (Mitscher *et al,* 1986).

It has been suggested that medicinal plants possess anticarcinogenic and antimutagenic activities are considered novel chemopreventive agents through the variety of mechanisms such as inhibition of genotoxic effects, induction of apoptosis, alteration of signal transduction, modulation of antioxidant activities, scavenging free radicals, and enhancing the activities of detoxification enzymes (Aruna *et al* , 1992, Govindasamy *et al,* 2012).

The National Cancer Institute has identified several commonly used herbs as possessing cancer-preventive properties. The list includes some spices of Labiatae family (basil, mints, oregano, rosemary, sage, and thyme); spices of the Zingiberaceae family (turmeric and ginger); and spices of the *Umbelliferae* family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, and parsley) (Caragay *et al* 1992).

In the present study, we aim to determine the antioxidant and antimutagenic potential of two commonly used spices of Apiaceae Family, viz Trachyspermum ammi (Ajwain) **and** ***Foeniculum vulgare*** **(Fennel) against induced mutagenesis in** ***Salmonella typhimurium*** **strains.**

OBJECTIVES

The proposed study is designed to-

1. Screen ajwain and fennel seed extracts and essential oil for their phytoconstituents and presence of bioactive compounds.
2. Determine the *in vitro* antioxidant activties of ajwain and fennel seed extracts and essential oil.
3. Determine the mutagenicity of ajwain and fennel seed extracts and essential oil in *Salmonella typhimurium* strains.
4. Determine the antimutagenic potential of ajwain and fennel seed extracts and essential oil against induced mutagenicity in *Salmonella typhimurium* strains by salmonella/microsome reversion assay/Ames test using the plate incorporation method.

PROPOSED METHODOLOGY

EXTRACTION

Plant material

The seed of fennel *(Foeniculum vulgare)* and ajwain (*Trachyspermum ammi)* would be purchased from NRCSS, Tabiji farm, Ajmer.

Preparation of extract

Methanolic and acetonic extracts of ajwain and fennel would be prepared by soxhlet extraction assembly (Javed *et al,* 2012). Aqueous extract would be prepared using mechanical shaker (Oyedemi *et al,* 2010*).*

Preparation of essential oil

The essential oil of ajwain and fennel would be obtained by hydrodistillation using a Clevenger-type hydrodistillation apparatus (Javed *et al* 2012).

PHYTOCHEMICAL ANALYSIS

Phytochemical analysis

Phytochemical tests for confirming the presence of carbohydrates, reducing sugars, tannins, flavonoids, saponins, phytosterols and fixed oils and fats in aqueous, acetonic and methanolic extracts of ajwain and fennel would be performed as following:

S.No.	Phytoconstituents	Tests	References
1.	**Alkaloids**	Mayer's Test Wagner's Test	(Evans, 1997) (Wagner, 1993)

		Hager's test	(Wagner *et al,* 1996)
2.	**Carbohydrates**	Molisch's test Barfoed's test Fehling's test for reducing sugars	(sofowara,1993)
3.	**Saponins**	Frothing test	(Kokate, 1999)
4.	**Phytosterols**	Libarman-Burchard's test Salkowski's Test	(Finar, 1986)
5.	**Phenols**	Ferric Chloride Test	(Mace, 1963)
6.	**Tannins**	Gelatin Test	(Evans, 1997)
7.	**Flavanoids**	Alkaline Reagent Test Shinoda Test	(Trease and Evans,2002) (Harborne, 1998)
8.	**Glycosides**	Legal's Test	(Evans, 1997)
9.	**Fixed oils and Fats**	Spot test Saponification test	(Kokate, 1999)

Estimation of total phenolic compounds

Polyphenolic compounds are known to have antioxidant activity and it is likely that the antimutagenic activity of the extracts is due to these compounds (Okudu *et al,* 1994). Hence, Estimation of total phenolic compounds would be carried out using following methods:

- **Total Phenolic Content** (Wolfe *et al,* 2003)
- **Total flavanoid content** (Ordon *et al, 2006)*
- **Total flavanols** (Kumaran and Karunakaran 2007).

- **Total proanthocyanidins** (Sun *et al,*1998)

Thin Layer Chromatography

The fraction of extracts and essential oil of ajwain (Ashnagar *et al,* 2011) and fennel (Muthanna *et al,* 2009) would be further purified by TLC.

GC-MS analysis

In our study we aim to investigate the antioxidant and antimutagenic potential of new variety of ajwain *(Trachyspermum ammi)* and fennel (*Foeniculum vulgare* Mill). For this purpose identification of all major bioactive compounds by **GC/MS** present in these spices is necessary (Anwar *et al,* 2008, Aprotosoaie *et al,* 2010, Goudarzi *et al,* 2011, Sathianarayanan *et al,* 2011,)

ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITY

In vitro antioxidant assays

Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds (Okudu *et al,* 1994). This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng *et al,* 2001) associated with lower occurrence and lower mortality rates of several human diseases (Anderson *et al,* 2001, Djeridane *et al,* 2006). Therefore, the extracts and essential oil of ajwain and fennel would be studied for their antioxidant potential through following *in vitro* model systems.

S.No.	Method	References
1.	DPPH (1, 1diphenyl2picryl hydrazyl) radical scavenging activity	Vilasrao *et al,* 2010
2.	Hydrogen peroxide radical scavenging activity	Ruch *et al,* 1989
3.	Ferric reducing/ antioxidant power (FRAP) assay	Benzie and Strain, 1999
4.	β-Carotene/Linoleic Acid Bleaching Assay.	Miraliakbari and shahidi, 2008
5.	ABTS radical scavenging assay	Re *et al,* 1999
6.	Nitric oxide radical inhibition activity	Garrat, 1964

Salmonella typhimurium strains

The tester strains *Salmonella typhimurium* viz. TA98, TA100 and TA102 would be obtained from microbial type culture collection and Gene bank, IMTEC (Institute of microbial technology), Chandigarh (INDIA).

Genetic analysis of strains

The tester strains would be analyzed for their genetic integrity and spontaneous mutation rate when frozen cultures are prepared. A strain check would be performed whenever an experiment is performed (Tejs, 2008). The steps given below would be followed for a complete strain check.

- Histidine dependence
- Biotin dependence
- Biotin and histidine dependence
- *rfa* marker
- Presence of plasmid pKM101 (ampicilline resistance)
- Spontaneous mutant frequency

Preparation of metabolic activation (S9) mix

The S9 mix would be prepared according to the recipe recommended by Maron and Ames in 1983 and Mortelmans and Zeiger in 2000. S9, a cell free fraction would be prepared by homogenization and centrifugation of rat liver at 9000×g for 10 min. and added when metabolic activation is required for indirect mutagens.

Mutagenicity test for seed extracts/essential oil

The Ames test would be performed by the plate incorporation method as described by Ames *et al,* in 1975 and revised by Maron and Ames in 1983 using *S. typhimurium* TA98, TA100 and TA102.

Antimutagenicity test by plate incorporation method

The salmonella/microsome reversion assay would be conducted using the plate incorporation procedure described by Ames *et al,* 1975 and revised by Maron and Ames (1983). *Trachyspermam ammi* (ajwain), and *Foeniculum vulgare* (fennel) seed extracts and essential oil would be tested for their antimutagenic properties against induced mutagenesis of 2-Aminofluorene (2-AF), Benzo[*a*]pyrene (B(a)P) and 7, 12-dimethylbenz (alpha) anthracene (DMBA) in *Salmonella typhimurium* strains TA98, TA100 and TA102.

Statistical analysis

All the experiments would be carried out in triplicates and the results would be expressed as mean ± standard deviation. The data would be further analyzed for statistical significance using analysis of variance (one way ANOVA).

PLAN OF WORK

EXTRACTION

Preparation of **aqueous, acetonic and methanolic extract** of ajwain and fennel seeds.

Extraction of **essential oil** of ajwain and fennel seeds.

PHYTOCHEMICAL ANALYSIS

Phytochemical analysis

Estimation of total phenolic compounds

TLC and **GC-MS** analysis for identification of **bioactive compounds**

ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITY

***IN VITRO* ANTIOXIDANT ACTIVTY**

- DPPH (1, 1diphenyl2picryl hydrazyl) radical scavenging activity
- Hydrogen peroxide radical scavenging activity
- Ferric reducing/ antioxidant power (FRAP) assay
- ABTS radical scavenging assay
- β-Carotene/Linoleic Acid Bleaching Assay.
- Nitric oxide radical inhibition activity

MUTAGENICITY of seed extracts and essential oil

ANTIMUTAGENIC POTENTIAL of seed extracts and essential oil

Salmonella/microsome reversion assay/Ames test – **Plate incorporation method**

***Salmonella typhimurium* strains**

- **TA98**
- **TA100**
- **TA102**

Mutagens

- **2-AF**
- **B(a)P**
- **DMBA**

Significance of the study

Based on the traditional use for long period of time, spices are often assumed to be safe. They play an important role in health services all over the world. By virtue of their powerful phytochemicals, herbs and spices are known to exhibit an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties that are believed to contribute to their anticarcinogenic and antimutagenic activities.

Use of antimutagens and anticarcinogens in everyday life has been suggested to be the most effective procedure for preventing human cancer and genetic diseases. These herbs and spices have components as bioactive compounds which act as strategy to block or reverse carcinogenesis at early stages. Moreover, they are considered to be an inexpensive, effective and easily applicable approach to control cancer. The wide spread medicinal, edible and herbal plants have been tested for their antimutagenic activity and proved to inhibit the mutagenic and/or carcinogenic effects of some chemical mutagens. In recent years, there has been a global surge in the popularity of herbal/traditional medicine, and currently there is enormous interest in developing new pharmaceutical products from such resources. Therefore, it can be suggested that *Trachyspermum ammi* (ajwain) *and Foeniculum vulgare* Mill (fennel) could serve as an important resource in developing new herbal pharmaceutical products.

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